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Hepatoprotective effects of lycopene against carbon tetrachloride-induced acute liver injury in rats

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ABSTRACT

Lycopene, the major carotenoid in tomatoes, is a known antioxidant that may lower oxidative stress biomarkers by a mechanism that is not fully elucidated. The intoxication of rats with carbon tetrachloride (CCl_4) resulted in significant histological hepatic degradation accompanied by a marked increase in reactive oxygen species (ROS) and in the number of apoptotic cells. The induced oxidative stress in turn results in a significant elevation of lipid peroxidation and H_2O_2 generation, together with a decrease in the concentration of reduced glutathione (GSH) and a significant reduction in activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S transferase (GST). CCl_4-intoxicated rats, pre-treated with lycopene, showed strongly reduced cell damage and ROS generation. The level of markers for hepatic integrity in lycopene pre-treated rats was close to the controls in the absence of CCl_4 treatment, indicating the protective effect of lycopene pre-treatment. In the same way, lycopene pre-treated rats significantly increased SOD, CAT, GPx, GST activities and GSH level. In addition, we measured an increased lipoxygenase (LOX) activity in CCl_4-intoxicated rats. This activity was reduced in lycopene pre-treated rats to values close to those observed in the controls, suggesting a potential pharmacological application of this dietary carotenoid.

1. Introduction

Lycopene is a carotenoid with a potent antioxidant activity, found in tomato and tomato products. The known protective effect of tomatoes against some types of pathologies mediated by oxidative processes has been attributed to the carotenoid constituents of the fruit, mainly lycopene (Abete et al., 2013). This compound acts as a powerful antioxidant and is an effective agent against chronic inflammatory-related diseases, including cardiovascular diseases and some types of cancer, which involve radical formation and cytokine production, being one of the main carotenoids in numerous medicinal plants and fruits (Igual, Garcia-Martinez, Camacho, & Martinez-Navarrete, 2013; Siriamornpurn, Kaisoon, & Meeso, 2012). Previous studies show that lycopene inhibits the inflammatory cascade in cultured cells (Hazewindus, Haenen, Weseler, & Bast, 2012; Ilahy, Hdider, Lenucci, Tlili, & Dalessandro, 2011; Yang et al., 2013) and that these chemopreventive effects of lycopene *in vitro* are because of its antioxidant efficiency and its capacity as a singlet oxygen and free radical scavenger (Atessian, Yilmaz, Karahan, Ceribasi, & Karaoglu, 2005). However, in human and animal trials using lycopene or lycopene-containing extracts, the low level of lycopene detected in tissues suggests that the metabolic products of lycopene may be responsible for its known bioactivity (Erdman, Ford, & Lindshield, 2009). The complexity of lycopene's antioxidant activity is demonstrated by its inhibitory effects on the growth of prostate cancer cell lines, This multifactorial mechanism

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includes the inhibition of cell cycle progression, induction of apoptosis, modulation of gap-junctional intercellular communication, inhibition of IGF-1 signal transduction, inhibition of IL-6 expression and induction of phase II detoxifying enzymes, among others (Hazai, Bikadi, Zsila, & Lockwood, 2006).

In addition to anticarcinogenic effects, lycopene has been found to have ameliorative properties against several toxic agents, such as pesticides, ionising radiation or potent oxidants like hypochlorous acid or mercuric chloride. Treatment with lycopene attenuated induced oxidative stress approaching the lipid peroxidation level, the SOD, CAT and GSH-Px activities and the GSH concentration to values near to the controls. However, the mechanisms through which lycopene exerts its antioxidant properties have not yet been elucidated (Deng, Xu, Liu, & Yang, 2012; Pennathur et al., 2010; Srinivasan, Devipriya, Kalpana, & Menon, 2009; Yonar & Sakin, 2011).

The main objective of this study is to further understand the mechanism of lycopene's antioxidant action by evaluating the protective effect of orally administered lycopene pretreatment on CCl_4-intoxicated rats. CCl_4 is a hepatotoxic agent used to induce liver injury in experimental animals to check the efficiency of potential hepatoprotective agents.

During the first phase of the CCl_4-induced hepatotoxicity, cytochrome P450 metabolises CCl_4 to two trichloromethyl radicals ($^{\cdot}CCl_3$ and $CCl_3OO^{\cdot}$) that are assumed to initiate free radical-mediated lipid peroxidation. This activity leads to the accumulation of oxidation products that cause membrane lipid peroxidation and cell necrosis (Basu, 2003). In the second phase, Kupffer cells are activated and begin producing proinflammatory mediators (Planagun et al., 2005). Studies in cultured rat hepatocytes have shown that carotenoids protect from CCl_4-induced injury by suppression of lipid peroxidation, thus improving cell survival (Kim, 1995); another study has shown that a direct and quick interaction between lycopene and the produced trichloromethyl peroxyl radical occurs *in vitro* (Yaping et al., 2002), however, the evaluation *in vivo* of the hepatoprotective properties of lycopene remain to be sufficiently studied. In this study, we use an animal model of hepatoxicity previously reported by us for the evaluation of *in vivo* antioxidant properties of xanthohumol (Pinto, Duque, Rodriguez-Galdón, Cestero, & Macias, 2012). In this experimental approach we have established measurements of the hepatoprotective efficiency of lycopene using oxidative stress markers in both biochemical and histological experiments.

2. Materials and methods

2.1. Chemicals

Lycopene (95%) and all chemicals used in our biochemical assays were obtained from Sigma–Aldrich Quimica (Madrid, Spain). All other chemicals were of analytical grade.

2.2. Animals and experimental design

Wistar rats (200–250 g) were maintained in standard environmental conditions, at 22 °C with a 12 h light/dark cycle, controlled humidity and air circulation and free access to food and water. Appropriate guidelines from the local animal ethics committee (Bioethics and Biosafety Committee of Extremadura University) were followed for all animal experiments.

The rats were randomly divided into ten groups with nine animals per group. In all cases, rats were treated with either lycopene or vehicle with an orogastric tube. Lycopene at the indicated concentration was prepared using lard from the fatback of Iberian pigs as vehicle and had a typical fatty acid profile of 31.5% saturated fatty acids, 57.9% monounsaturated fatty acids and 10.5% polyunsaturated fatty acids (Ventanas, Ventanas, Jurado, & Estevez, 2006). Samples were always administered at 30 °C to maintain the lard in liquid state. The experimental design is described in Table 1. Group 0 corresponds to rats without treatment. Group I was the normal control that was given vehicle alone (2.0 mL/kg b.w.) each day for 8 days. Group II was treated Group I, but on the 7th day, a 2.5 mL/kg b.w. dose of CCl_4 was intraperitoneally (i.p.) injected into each animal. Groups III, IV and V were treated with 2.0 ml/kg b.w. of lycopene dissolved in lard at dose levels of 0.35, 0.65 and 1.30 mg/kg b.w., respectively, each day for 6 days; on the 7th day, a single dose of CCl_4 (2.5 mL/kg i.p.) was administered. The range of doses used in this work was established by a previous study on the effect of lycopene (data not shown). Groups IIIc, IVc and Vc were controls for groups III, IV and V and consisted of rats that were pre-treated with the same lycopene doses but not administered the CCl_4 treatment. After 24 h, each rat was anesthetised with chloroform to draw blood and remove the liver. Blood samples were collected by cardiac puncture and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 600×*g* for 15 min and analysed for GOT, GPT and LDH. The livers were excised, homogenised and assayed for CAT, SOD and GSH-dependent enzyme activity, GSH, thiobarbituric acid reactive substances (TBARS), H_2O_2 levels and pathological histology, according to the procedures described below.

2.3. Hepatic homogenate preparation

The liver was homogenised in a solution of 10 mM KCl, 1 mM ethylenediaminetetraacetic acid and 100 mM phosphate buffer (pH 7.4). The homogenate was centrifuged at 12,000×*g* for 60 min. The supernatant was used to assay enzymatic markers of oxidative stress, including GPx, GST, GRase, SOD and CAT activities. Also we determined GSH, TBARS, H_2O_2 levels and the total protein content.

2.4. Histological evaluation of liver damage

Liver tissues were fixed in a fresh solution of 4% paraformaldehyde (pH 7.4) at 4 °C for 24 h. After dehydration with an ethanol solution and clearing with xylene, the liver tissues were embedded in paraffin and sectioned at 5 μm. These sections were then stained with Harris' hematoxylin (5 min at room temperature) and Eosin Yellowish (0.5%, 2 min at room temperature) to evaluate the histological alterations using optical microscopy (Olympus BX51. Olympus, Tokyo, Japan).

To investigate the effect of oxidative stress on the polar/non-polar lipid ratio, samples were stained with Nile Red at 0.5 μg/mL. This compound is a fluorescent lipophilic dye characterised by an emission shift from red to yellow, according to